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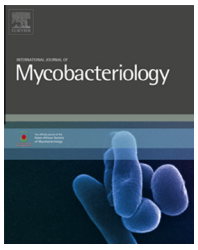

Cloning, expression, and purification of recombinant protein MPT-64 from a virulent strain of *Mycobacterium bovis* in a prokaryotic system

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ABSTRACT

Objective: Tuberculosis (TB) is a zoonotic infectious disease common to humans and animals that is caused by the rod-shaped acid-fast bacterium *Mycobacterium bovis*. Rapid and sensitive detection of TB is promoted by specific antigens. Virulent strains of the TB complex from *M. bovis* contain 16 regions of difference (RD) in their genome that encode important proteins, including major protein of *Mycobacterium Tuberculosis* 64 (MBT-64, which is a primary immune-stimulating antigen encoded by RD-2. In this study, we cloned, expressed, and purified MPT-64 as a potent *M. bovis* antigen in a prokaryotic system for use in future diagnostic studies.

Methods: The antigenic region of the *Mpt64* gene was investigated by bioinformatics methods, cloned into the PQE-30 plasmid, and expressed in *Escherichia coli* M15 cells, followed by isopropyl β-D-1-thiogalactopyranoside induction. The expressed protein was analyzed sodium dodecyl sulfate polyacrylamide gel electrophoresis and purified using a nickel-affinity column. Biological activity was confirmed by western blot using specific antibodies.

Results: Our data verified the successful cloning of the *Mpt64* gene (687-bp segment) via the expression vector and purification of recombinant MPT-64 as a 24-kDa protein.

Conclusion: These results indicated successful expression and purification of recombinant MPT-64 protein in a prokaryotic system. This protein can be used for serological diagnosis, improved detection of pathogenicity and non-pathogenicity between infected cattle, and for verification of suspected cases of bovine TB.

Conflicts of interest

The authors have no conflicts of interest to declare.

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